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Porcine circovirus type 2 (PCV2) enteric disease: An independent condition or part of the systemic disease?

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ABSTRACT

Intestinal disorders in growing and finishing pigs have been associated with several infectious agents, including Porcine circovirus type 2 (PCV2). This virus has been mainly related with PCV2-systemic disease (PCV2-SD); nevertheless, some authors have suggested a possible restricted intestinal infection of this virus associated with enteric clinical signs. This condition has been referred as PCV2-enteric disease (PCV2-ED). The present study analysed retrospectively, from a pathological point of view, the relation between intestinal disorders and PCV2 infection in nursery and growing-finishing pigs. Among the 96 selected pigs suffering from enteric disease and submitted for necropsy between 1998 and 2011, the most prevalent enteric lesions were catarrhal enteritis/colitis (77.1%), followed by fibrinous lesions (11.5%), granulomatous inflammation (4.2%) and other lesions such as haemorrhages or ulceration (4.2%). Seventy-two pigs (75%) were positive for PCV2 by *in situ* hybridization (ISH).

Among positive pigs for PCV2 ISH, 39 animals suffered from PCV2-SD and 33 had no lymphoid lesions but low amount of viral nucleic acid in several lymphoid tissues, therefore, these animals did not qualify for PCVD-ED. In conclusion, all animals with enteric disorders that were positive to PCV2 by ISH had evidence of viral systemic infection. These results suggest that PCV2-ED is probably a negligible condition and PCV2 mainly contributes to enteric clinical disorders in relation to PCV2-SD occurrence.

1. Introduction

Intestinal disorders in growing-finishing pigs are a common problem in most commercial farms and significantly limit the efficiency and profitability of swine production globally. Clinical presentation consists of diarrhoea, poor growth and variable mortality. Intestinal disorders in pigs older than four weeks of age can be produced by several infectious agents such as rotaviruses, some coronaviruses, porcine circovirus type 2 (PCV2), *Escherichia coli*, *Brachyspira* spp., *Salmonella* spp., *Yersinia* spp., *Lawsonia intracellularis*, *Oesophagostomum dentatum* and *Trichuris suis*, among others. However, most cases featuring postweaning diarrhoea and colitis are due to concurrent aetiologies (Thomson and Friendship, 2012).

PCV2 is recognized as one of the most important viruses causing severe economic impact in the swine industry worldwide and it has been described to cause different conditions depending on the virus, host immunity,

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co-infections and other environment characteristics (Segalés et al., 2005; Madec et al., 2008). These diseases are grouped under the name porcine circovirus diseases (PCVDs). PCVDs include PCV2-systemic disease (PCV2-SD, formerly known as postweaning multisystemic wasting syndrome, PMWS), porcine dermatitis and nephropathy syndrome (PDNS), PCV2 reproductive disease (PCV2-RD), PCV2-lung disease (PCV2-LD) and PCV2-enteric disease (PCV2-ED) (Segalés, 2012).

Diarrhoea is a feature of some PCVDs and has been described in cases of PCV-SD and PCV-ED (Kim et al., 2004; Segalés et al., 2004; Opriessnig et al., 2007). PCV2-SD occurs in pigs between one and six months of age with the highest number of cases occurring between two and three months (Segalés and Cortey, 2010). It is a multifactorial disease, in which infection by PCV2 is essential but other factors are usually required for its complete expression (Segalés et al., 2005; Madec et al., 2008). From a clinical point of view, there may be a potential diagnostic overlapping between PCV2-SD and PCV2-ED since diarrhoea can be easily present in cases of the systemic disease (Opriessnig et al., 2007). However, some authors have suggested that PCV2-ED could be a distinct clinical manifestation of PCV-2 (Kim et al., 2004). To differentiate between these two conditions, histopathological findings and examination not only of gut but also lymphoid tissues is crucial. PCV-SD is diagnosed when the following are present: (a) clinical signs consisting of weight loss and paleness of skin (respiratory and/or digestive clinical signs may be present as well), (b) moderate to severe lymphocyte depletion with granulomatous inflammation of lymphoid tissues or other organs, and (c) moderate to high amount of PCV2 in damaged tissues. On the other hand, PCV-ED is diagnosed when (a) pigs suffer from diarrhoea; (b) a granulomatous enteritis and lymphocyte depletion is observed microscopically in Peyer's patches, but not in other lymphoid tissues, and (c) a moderate to high amount of PCV2 is detected in intestinal mucosa or Peyer's patches without detection in other lymphoid tissues (Segalés, 2012).

From a case definition point of view, PCV2-ED and PCV2-LD share a common characteristic, which is the presence of lesions and PCV2 antigen/genome in intestines and lung, respectively, in absence of lesions and viral detection in systemic locations (Segalés, 2012). However, the specific study of PCV2-LD in a set of 317 pigs suffering from respiratory disease, indicated that this condition is probably negligible and PCV2 mainly contributes to respiratory disease in relation to PCV2-SD occurrence (Ticó et al., 2013). This type of assessment has not been yet conducted for PCV2-ED. Therefore, in order to clarify some points of PCV2-ED, the present study aimed to investigate the prevalence of PCV2 infection in growing-to-finishing pigs with diarrhoea in Spain and assess the potential overlap between PCV-ED and PCV2-SD.

2. Materials and methods

2.1. Animals

In this study, 96 pigs (3.7%) were selected from a total of 2566 pigs that were submitted for necropsy between 1998 and 2011 to the Veterinary Pathology Diagnostic Service at the Veterinary School of Barcelona (Spain). Animal selection criteria included the following four features: (a) older than four weeks of age (nursery, growing and finishing pigs), (b) clinical digestive signs, (c) availability of intestinal and lymphoid tissues to assess histopathological lesions and to detect PCV2 DNA by *in situ* hybridization (ISH) and (d) presence of microscopic lesions in the intestine.

2.2. Histopathology

Formalin-fixed, paraffin-embedded samples of intestines (portions of jejunum, ileum and colon) and lymphoid organs (mesenteric and inguinal superficial lymph nodes, and tonsil) corresponding to the selected 96 pigs were included in this study. All samples were analysed by the same pathologist. Classification of lesions was performed in regards of gross and/or microscopic findings; hence, diagnostic criteria were as follows. Catarrhal enteritis or colitis (CE/CC) was consistent with serous or mucous intestinal content in the lumen and moderate to severe lymphoplasmacytic infiltration was observed microscopically within the intestinal mucosa. Granulomatous enteritis or colitis (GE/GC) was assessed when multifocal aggregates of macrophages (granulomas), with or without multinucleated giant cells or intracytoplasmic inclusion bodies, and lymphoid depletion were observed in the mucosa and/or Peyer's patches. Haemorrhagic enteritis (HE) was classified when haemorrhagic content was observed in the small intestine. Fibrinous enteritis or colitis (FE/FC) consisted of accumulation of fibrin on the mucosa of caecum or colon. Ulcerative colitis (UC) was characterized by multifocal to coalescing ulceration of the large intestinal mucosa. Mucous-haemorrhagic colitis (MHC) consisted of mucous and haemorrhagic content in the caecal or colonic lumen. Lesions were characterized as "single" or "combination" when one or more lesion types were observed, respectively.

Diagnosis of PCV2-SD was established following internationally accepted criteria previously described (Segalés, 2012). Regarding the PCV2-ED, criteria proposed in the same article were used: (1) lympho-histiocytic to granulomatous enteritis and lymphocyte depletion with granulomatous inflammation in Peyer's patches, (2) positive ISH result for PCV2 in the intestines, and (3) absence of PCV2 genome and histopathologic PCV2-SD lesions in other lymphoid tissues (Segalés, 2012).

2.3. In situ hybridization

The presence of PCV2 in tissues was determined by an ISH technique (Rosell et al., 1999), which was performed on lymph node, tonsil and intestines, using a 41 bp digoxigenin labelled DNA probe corresponding to ORF1 of PCV2 (DIG-5′-CCT TCC TCA TTA CCC TCC TCG CCA ACA ATA AAA TAA TCA AA-3′). Quantification of PCV2 nucleic acid detection was made according to a subjective evaluation of the percentage of positive cells from the totality of the tissue submitted in the slide: – (negative), + (less than 10%), ++ (between 10% and 50%) and +++ (more

than 50%). These samples were analysed in a blinded fashion manner by the same pathologist.

3. Results

3.1. Animals

All pigs came from 45 different Spanish farms, each one with its own particular management, system production and size. From 96 pigs studied, 80 animals (83.3%) were necropsied between 1998 and 2006 (pre-PCV2 vaccination) and only 18 pigs (18.7%) were submitted from 2007 to 2011. Forty-four animals were among four and eight weeks old (nursery pigs) and fifty-two were older than eight weeks (growing-finishing pigs).

3.2. Lesions and PCV2 detection

A summary of enteric lesions and PCV2 detection is shown in Table 1, where numbers of pigs with single or combined lesions are given in detail. The majority of pigs displayed a "single" lesion (97.9%), while only two pigs had a combination of two types of lesions (2.1%). The most common lesion was colitis, observed in 53 pigs (55.2%), followed by enterocolitis in 31 animals (32.3%) and enteritis in 12 pigs (12.5%). Catarrhal lesions were the most prevalent with 74 pigs affected (77.1%), followed by fibrinous lesions observed in 11 animals (11.5%), granulomatous inflammation in four (4.2%) and other lesions, such as haemorrhages, ulceration or mucohaemorrhagic exudate, in seven pigs (7.2%). No lesions compatible with proliferative ileitis were observed. Focusing on the age of animals, catarrhal lesions were slightly more frequent in nursery pigs (84.2%) compared to growing-finishing animals (75.3%). In contrast, fibrinous lesions were more prevalent in the growing-finishing (76.9%) than in the nursery period (23,1%). Only nine animals (9.4%) were submitted between 2007–2011 (post-PCV2 vaccination).

In regards of ISH positivity for PCV2 among the intestinal samples, enterocolitis was positive in 77.4% of the animals (24/31) and 45.2% of these cases (14/31) had a diagnosis of PCV2-SD. Colitis were positive in 77.4% (41/53) and 37.7% (20/53) corresponded to a PCV2-SD. Enteritis cases were positive to PCV2 in 58.3% of cases (7/12) and 41.6% of animals (5/12) where diagnosed as a PCV2-SD. Besides, there was a group of animals where PCV2 was not detected in the intestine, but yielded positive in lymph nodes and tonsils; this occurred in 17.7% of pigs (17/96); from them, 6 corresponded with a PCV-SD diagnosis.

Taking only into account the positivity for PCV2 in the intestinal lesions, from the 96 pigs with intestinal lesions, 72 (75%) were positive for PCV2 ISH and 24 pigs were negative for this virus in intestine and lymphoid tissues (Fig. 1). Thirty-nine pigs out of the 72 PCV2 ISH positive (54.7%) suffered from PCV2-SD. The remaining 33 pigs with PCV2 ISH positivity in the intestine had also low or moderate quantity of viral genome in lymph nodes and tonsil. Therefore, none of the studied animals qualified for a PCV2-ED diagnosis.

4. Discussion

Intestinal disorders are a common health problem in postweaning pigs. However, in the present work, considering the selection criteria, a small percentage of animals were investigated compared to other similar studies performed in pigs with respiratory disorders (Ticó et al., 2013). Several factors could be involved in this low casuistic. First, the necessary presence of gross or microscopic intestinal lesions to be included in the study, as some intestinal disorders such as osmotic or dietary diarrhoeas do not produce lesions. Second, most intestinal

Table 1
Summary of enteric lesions in relation with *in situ* hybridization for PCV2 (ISH-PCV2).

Single lesion or combination	Number of pigs	PCV2-SD diagnosis		Positive ISH-PCV2 without PCV2-SD diagnosis		Negative ISH-PCV2[+]
		ISH-PCV2 positive	ISH-PCV2 negative[*]	ISH-PCV2 positive	ISH-PCV2 negative[*]	
Enteritis	**12**	**4**	**1**	**2**	**–**	**5**
Catarrhal	9	3	–	2	–	4
Granulomatous	2	1	1	–	–	–
Haemorrhagic	1	–	–	–	–	1
Colitis	**53**	**17**	**3**	**16**	**5**	**12**
Catarrhal	44	15	3	11	4	11
Fibrinous	6	2	–	3	–	1
Ulcerative	2	–	–	2	–	–
Muco-haemorrhagic	1	–	–	–	1	–
Enterocolitis	**31**	**12**	**2**	**4**	**6**	**7**
Catarrhal	21	7	–	3	4	7
Fibrinous	5	2	1	–	2	–
Granulomatous	2	1	–	1	–	–
Catarrhal and fibrinous	1	–	1	–	–	–
Ulcerative	1	1	–	–	–	–
Other	1	1	–	–	–	–
Total	**96**	**33**	**6**	**22**	**11**	**24**

PCV2-SD: porcine circovirus type 2 systemic disease.
[+] ISH-PCV2 was negative in all the tissues analysed.
[*] ISH-PCV2 was negative in the intestine but positive in lymph nodes and tonsil.

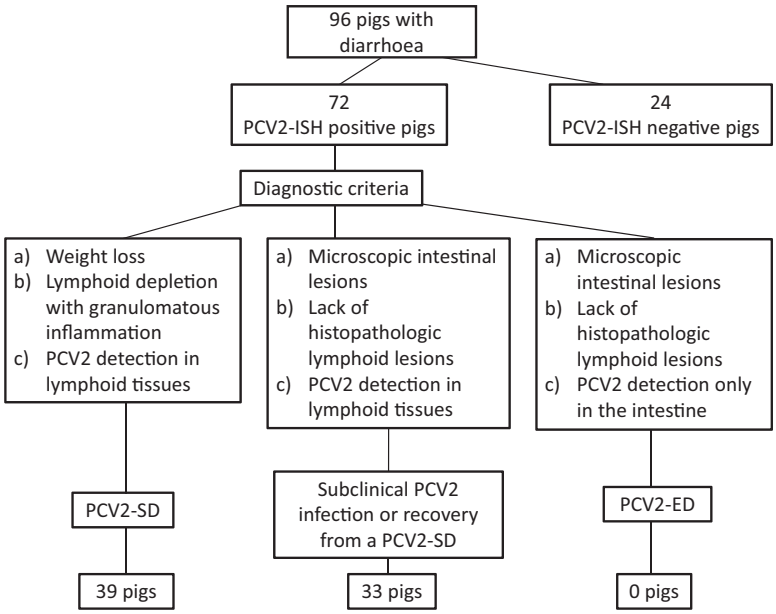

Fig. 1. Flow chart describing the selection and diagnostic criteria for PCV2 infected pigs included in this study. PCV2, porcine circovirus type 2; PCV2-SD, PCV2-systemic disease; PCV2-ED, PCV2-enteric disease.

disorders are usually treated with antibiotics or diet restriction and no animals are submitted for necropsy unless there is no recovery of clinical signs. And third, casuistic of digestive problems in nursery and growing-fattening pigs were of lower prevalence than respiratory disorders in the study period.

Different agents have been involved, including infectious and non-infectious factors, in intestinal disorders in growing-fattening pigs (Thomson and Friendship, 2012). Although bacteriological analyses were not performed in the studied cases, from a pathological point of view, intestinal lesions are usually related with specific infectious agents (Segalés and Martínez, 2013). The most prevalent intestinal lesion was catarrhal inflammation of different parts of the intestine. CE is usually associated with colibacillosis, or less frequently with yersiniosis; on the other hand, CC is generally related with the infection of some species of *Brachyspira*. However, other non-infectious factors, such as the composition of diet, could also be involved. Another less prevalent lesion was FE/FC which is generally associated with *Salmonella* spp. infections. Other type of lesion, such as HE and UC or MHC, usually associated with clostridiasis or swine dysentery, appeared in a very small number of cases. Surprisingly, only four pigs had granulomatous enteritis (highly suggestive of PCV2 infection) compared to the high rate of detection of this virus in the intestine or other organs of these animals. These findings suggest that intestinal disorders exclusively attributed to PCV2 infection in the intestine could be low, but this virus may predispose to opportunistic bacterial overgrowth or dysbacteriosis.

PCV2 has been included in the list of agents involved in the intestinal disorders of these animals (Kim et al., 2004; Segalés et al., 2004; Opriessnig et al., 2007). Evidence of immunosupression in PCV2 infected pigs has extensively been reported in regards of several features; for example, the lymphoid depletion observed in infected pigs and the association of PCV2-SD with opportunistic pathogens (Carrasco et al., 2000; Segalés and Mateu, 2006; Zlotowski et al., 2006). Another virus associated with immunosuppression is Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) (Zimmerman et al., 2012). In the present study, the presence of this virus in lung and lymphoid tissues was analysed by immunohistochemistry in 66 from the 96 pigs, but only 8 were positive (data not shown). In contrast, the present results showed a high prevalence of PCV2 infection in postweaning pigs with digestive disorders, reinforcing the hypothesis of altered immune response in animals infected by this virus. In this way, only a small percentage of animals corresponded to post-PCV2 vaccination period.

The diagnostic overlapping between PCV2-SD and PCV2-ED has been previously discussed (Opriessnig et al., 2007; Segalés, 2012). Kim et al. (2004) proposed that PCV2-associated enteritis (equivalent to PCV2-ED) should be differentiated from PCV2-SD based on clinical and histopathological criteria. However, in the present study, most of pigs showing clinical intestinal disorders with PCV2 involvement were histopathologically diagnosed as PCV2-SD or suffered from subclinical systemic infection of the virus. Moreover, the scenario in which PCV2 was present in intestine but not in lymphoid tissues, as requested to diagnose PCV2-ED (Segalés, 2012), did not occur in any case; so that, PCV2-ED as specific clinicopathological entity was absent in the current study. These results coincide with those published by Ticó et al. (2013); these authors studied the role of PCV2 associated lung

disease (PCV2-LD) in cases of respiratory disease of nursery and growing-finishing pigs, but PCV2 infection exclusively restricted to the lung was not found in any case. All these results support the idea of PCV2 as a systemic pathogen, rather than virus able to cause a facultative local infection. Although, concomitant viral or bacterial agents could be present in the intestinal studied cases (worsening clinical signs), the amount of PCV2 detected in intestines was never higher than the one detected in lymphoid lesions of the same animal. Moreover, in those cases in which PCV2-ED was potentially suspected (not diagnosed as PCV2-SD), the viral load in intestinal and lymphoid tissues was lower than the amount of virus detected in PCV-SD cases. In fact, obtained findings in pigs which were non-PCV2-SD, but PCV2-infected, suggest a PCV2 systemic infection which can be interpreted as: (1) subclinical PCV-2 systemic infection, being other pathogens the major contributors of diarrhoea, or (2) recovery phase from an old PCV2-SD infection.

5. Conclusions

The results obtained in this study suggest that PCV2 is frequent in cases of intestinal disorders in nursery and growing-finishing pigs. From a diagnostic point of view, the present retrospective study shows that the casuistic of enteric disorders cases that could be categorized in the group of PCV2-ED was non-existent in contrast to other published data (Kim et al., 2004; Opriessnig et al., 2007), and PCV2 mainly contributes to enteric clinical disorders in relation to PCV2-SD occurrence.

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